

A tarantula peptide against pain via ASIC1a channels and opioid mechanisms

Michel Mazzuca^{1,4}, Catherine Heurteaux^{1,4}, Abdelkrim Alloui^{2,4}, Sylvie Diochot¹, Anne Baron¹, Nicolas Voilley¹, Nicolas Blondeau¹, Pierre Escoubas¹, Agnès Gélout², Anny Cupo¹, Andreas Zimmer³, Anne M Zimmer³, Alain Eschalier² & Michel Lazdunski¹

Psalmotoxin 1, a peptide extracted from the South American tarantula *Psalmopoeus cambridgei*, has very potent analgesic properties against thermal, mechanical, chemical, inflammatory and neuropathic pain in rodents. It exerts its action by blocking acid-sensing ion channel 1a, and this blockade results in an activation of the endogenous enkephalin pathway. The analgesic properties of the peptide are suppressed by antagonists of the μ and δ -opioid receptors and are lost in *Penk1*^{-/-} mice.

Improving the treatment and the prevention of acute and chronic pain is an important goal for modern medicine. Ion channels have recently become very attractive targets in the search of new analgesics¹.

Acid-sensing ion channels (ASICs) are expressed throughout the central and peripheral nervous systems^{1–5}. ASIC1a, ASIC1b, ASIC2b and ASIC3 are abundantly expressed in small DRG neurons, where ASIC3 is predominantly implicated in a variety of pain sensations^{6–8}. ASIC1a, ASIC2a and ASIC2b are abundant in brain and spinal cord neurons^{2–8}. The most potent pharmacological agent for ASIC channels is psalmotoxin 1 (PcTx1). This peptide, which was isolated from a tarantula venom⁹, potently and specifically inhibits homomeric ASIC1a channels. PcTx1 is not lethal, even at high concentrations, and could be a candidate for therapeutic applications. PcTx1 blocks ASIC1a channels in brain neurons⁹, as well as in nociceptors¹⁰. It also blocks ASIC1a channels in spinal neurons (see Supplementary Results and Supplementary Fig. 1 online), which permits the use of intrathecal injections.

We first evaluated the effects of PcTx1 on behavioral reactions in acute pain models (see Supplementary Methods online). In tail immersion and hot plate tests, a large antinociceptive effect was observed following both intrathecal and intracerebroventricular (ICV) injections of PcTx1 (Fig. 1). Intraperitoneal or subcutaneous injections had no effect, which was expected, as PcTx1 is a peptide. Morphine induced an antinociceptive effect very similar to that of PcTx1 (Fig. 1a,b,d). A similar antinociceptive effect was also produced by intrathecal injection of specific antisense nucleotides (Fig. 1c) that have previously been used successfully¹¹ to eliminate ASIC1a expression in the retina. We used a formalin test to investigate the PcTx1 effects (intrathecal) in

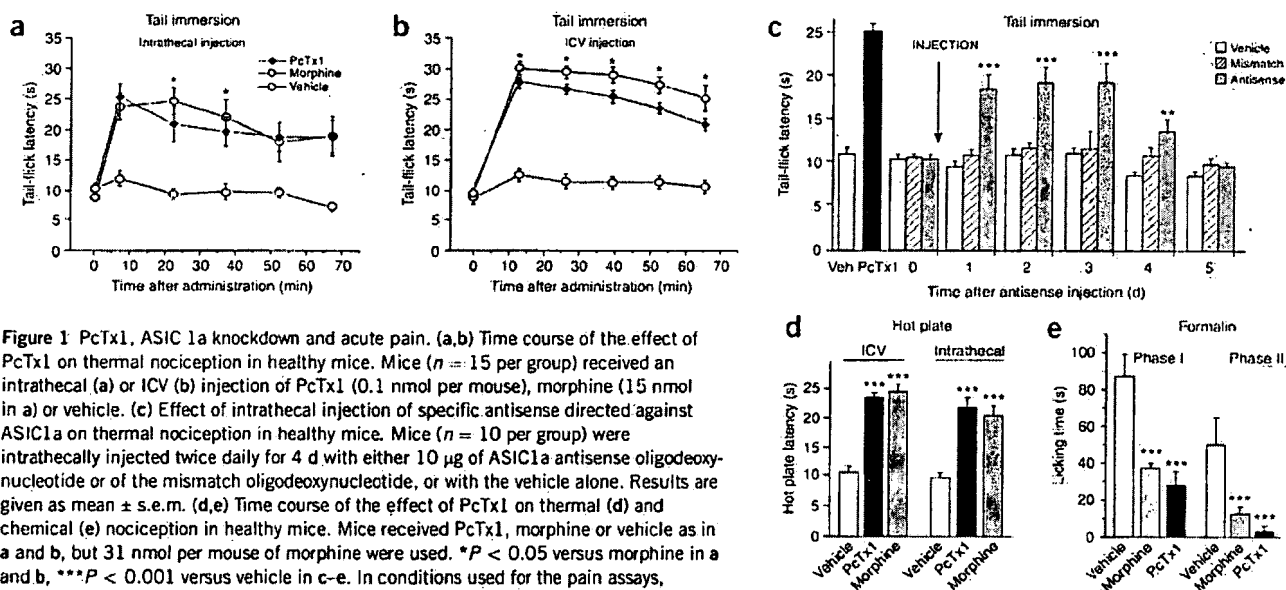
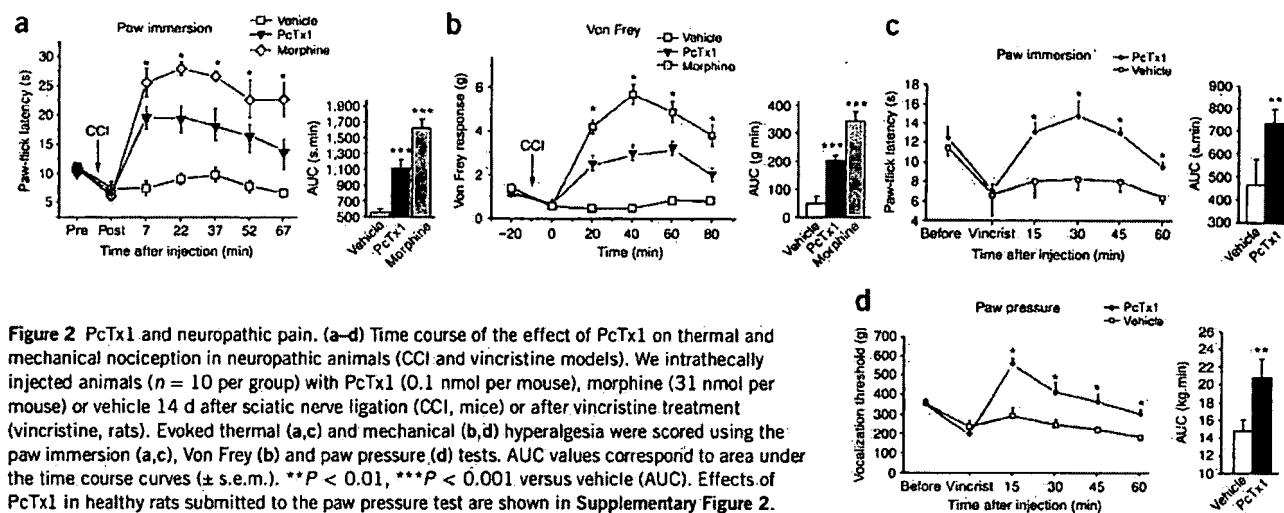


Figure 1 PcTx1, ASIC 1a knockdown and acute pain. (a,b) Time course of the effect of PcTx1 on thermal nociception in healthy mice. Mice ($n = 15$ per group) received an intrathecal (a) or ICV (b) injection of PcTx1 (0.1 nmol per mouse), morphine (15 nmol in a) or vehicle. (c) Effect of intrathecal injection of specific antisense directed against ASIC1a on thermal nociception in healthy mice. Mice ($n = 10$ per group) were intrathecally injected twice daily for 4 d with either 10 μ g of ASIC1a antisense oligodeoxynucleotide or of the mismatch oligodeoxynucleotide, or with the vehicle alone. Results are given as mean \pm s.e.m. (d,e) Time course of the effect of PcTx1 on thermal (d) and chemical (e) nociception in healthy mice. Mice received PcTx1, morphine or vehicle as in a and b, but 31 nmol per mouse of morphine were used. * $P < 0.05$ versus morphine in a and b, *** $P < 0.001$ versus vehicle in c–e. In conditions used for the pain assays, PcTx1, unlike morphine, had no effect on motor behavior (Supplementary Fig. 7).

¹Institut de Pharmacologie Moléculaire et Cellulaire, Centre National de la Recherche Scientifique (CNRS), Institut Paul Hamel, Université de Nice-Sophia-Antipolis, 660 Route des Lucioles 06560 Valbonne, France. ²Institut National de la Santé et de la Recherche Médicale, Unité Mixte de Recherche 766, Faculté de Médecine, CHU, Clermont-Ferrand, France. ³University of Bonn, Department of Molecular Psychiatry, Sigmund-Freud-Strasse 25, 53105 Bonn, Germany. ⁴These authors contributed equally to this work. Correspondence should be addressed to M.L. (lpmc@ipmc.cnrs.fr).

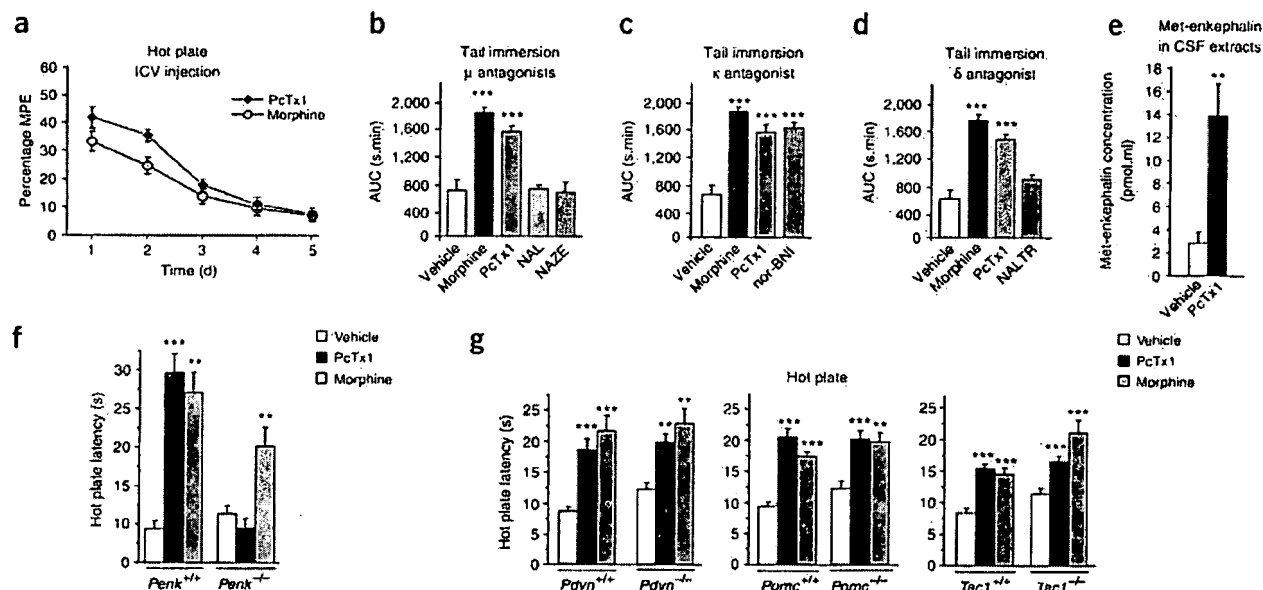
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irritant chemical nociception (acute, phase I) and in inflammation (late, phase II) (Fig. 1e). In phase I (0 to 5 min), morphine and PcTx1 had comparable antinociceptive effects. In phase II (from 30 to 40 min), the score reduction induced by PcTx1 was even higher than that induced by morphine ($P < 0.05$). These three acute pain models are indicative of a potent antinociceptive effect of PcTx1.

We then analyzed the effects of PcTx1 in two persistent pain models (see Supplementary Methods). Mononeuropathy caused by a chronic constriction injury (CCI) of sciatic nerve in rat produced a hyperalgesic

state and tactile allodynia (Fig. 2a,b). Similar to morphine, PcTx1 reversed both the thermal hyperalgesia and the tactile allodynia. On healthy rats, PcTx1 only slightly increased the mechanical threshold (Supplementary Fig. 2 online). We also assessed PcTx1 action using a mouse vincristine-induced neuropathy model (see Supplementary Methods). Vincristine is an anticancer drug that is unfortunately associated with peripheral neuropathy. Vincristine induced hypersensitivity to heat (Fig. 2c) and to mechanical stimuli (Fig. 2d), which was completely abolished by PcTx1 (Fig. 2c,d).



Because there is no additivity between the analgesic effects of morphine and PcTx1 (Supplementary Fig. 3 online) and because PcTx1 treatment, similar to morphine treatment, results in tolerance (Fig. 3a), we asked whether the analgesic ICV effect of PcTx1 was inhibited by naloxone, a nonspecific antagonist of the opioid receptors, by naloxonazine, a specific antagonist of μ 1-opioid receptors, by naltrindole, a δ -receptor antagonist, and by nor-binaltorphimine (nor-BNI), a κ -opioid receptor antagonist, both without any effect by themselves (Supplementary Fig. 4 online). The inhibitory effects of naloxone, naloxonazine and naltrindole indicated that PcTx1 produces its antinociceptive effect via the stimulation of μ - and δ -opioid receptors (Fig. 3b–d). ASIC1a was coexpressed with Met-enkephalin in the dorsal horn neurons (Supplementary Fig. 5 online). Enkephalins, endogenous opioid peptides, are potent ligands of both μ and δ receptors¹², and are involved in the analgesic effect of PcTx1. ICV injection of PcTx1 (like injection of ASIC1a antisense nucleotides, Supplementary Fig. 6 online) increased Met-enkephalin levels in the cerebrospinal fluid (Fig. 3e), and the analgesic effect of PcTx1 was lost in mice deficient for the *preproenkephalin* gene (*Penk1*^{−/−} mice) (Fig. 3f), but not in mice lacking either the *pro-opiomelanocortin- α* (*Pomc*^{−/−}) or *prodynorphin* (*Pdyn*^{−/−}) genes, or in mice deficient for *tachykinin* (*Tac1*^{−/−}) (Fig. 3g).

These results suggest that the ASIC1a channel is an important molecular target for treating both acute and neuropathic pain and that PcTx1 itself could be a new specific analgesic drug working upstream of the opiate receptors. Being a peptide, PcTx1 would have to be injected intrathecally to patients, similarly to ω -conotoxin MVIIA, a peptide originating from cone snail venom, which blocks high-voltage activated Ca²⁺ channels and has been approved for use in human patients¹³. Knowing that PcTx1 has analgesic effect and that it does not show some of the secondary effects of morphine (Supplementary Fig. 7 online) will also encourage the search for nonpeptidic blockers of the ASIC1a channel, which can be taken orally. Our work also highlights the connection between ASIC1a and the endogenous opioid system (Fig. 3). ASIC1a blockade leads to the activation of the enkephalin system, but the mechanistic details by which this happens are not yet known. Notably, ASIC1a is also modulated by peptides of the FMRF family^{5,8,14}, which are abundant in brain and spinal cord and have anti opioid properties¹⁵, and is inhibited by PcTx1, which displays opioid-like properties against pain. It is thus tempting to correlate these observations and to suggest that ASIC1 channels are at a crossroad between the enkephalin and the FMRF-related peptides pathways. It is also tempting to suggest that FMRF-like peptides negatively control the antinociceptive opiate

pathway through the ASIC1a channel. This negative control would then disappear when ASIC1a is blocked by PcTx1. Hopefully, this may open a new avenue of research into the endogenous opioid mechanisms in nociception.

Note: Supplementary information is available on the Nature Neuroscience website.

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AUTHOR CONTRIBUTIONS

M.M. and C.H. did the initial observations and conducted a large part of the experiments, analyzed the data and participated to the preparation of the manuscript. A.A., S.D., A.G. and A.E. conducted pain experiments both with PcTx1 and the antisense strategy. A.B. conducted localization and electrophysiological experiments and helped in the preparation of the manuscript. P.E. produced and provided PcTx1. N.V. was responsible for the localization and colocalization experiments. N.B. was associated with experiments using opiate antagonists. A.C. measured met-enkephalin concentrations in the cerebrospinal fluid. A.Z. and A.M.Z. provided the *Enk*^{−/−}, *Dyn*^{−/−} and *Tac1*^{−/−} mice. M.L. supervised the project and wrote the manuscript.

COMPETING INTERESTS STATEMENT

The authors declare no competing financial interests.

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